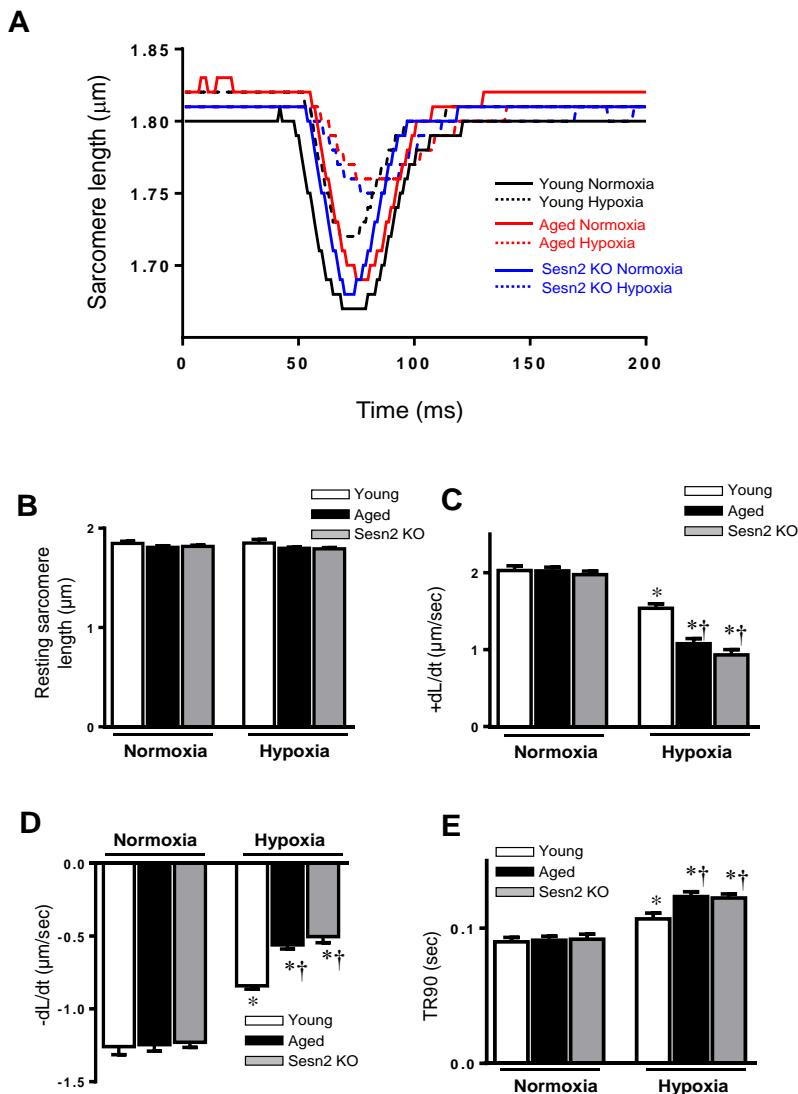
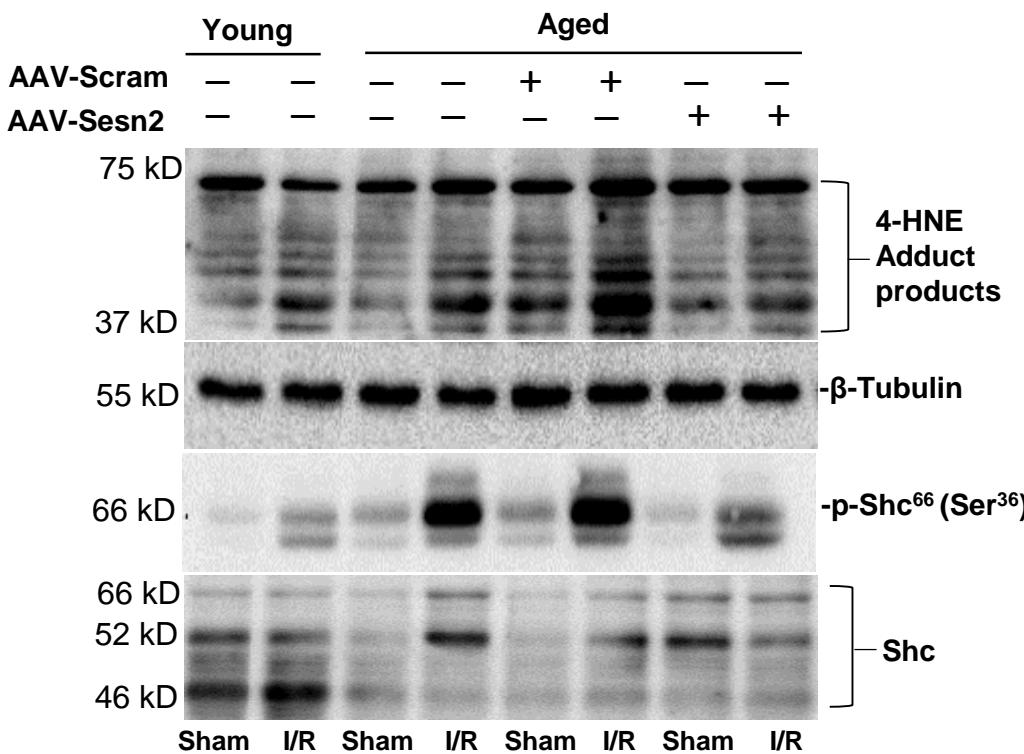


Sestrin2 Prevents Age-related Intolerance to Ischemia and Reperfusion Injury via Modulating Substrate Metabolism

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Suppl Figure 1. Contractile properties of cardiomyocytes from young, aged and Sesn2 KO hearts. (A) Representative sarcomeric length change recordings are shown during contractions stimulated at a frequency of 0.5 Hz. Recordings are shown from young, aged and Sesn2 KO cardiomyocytes for both normoxic and hypoxic conditions. (B) Resting sarcomere length was unchanged for young, aged and Sesn2 KO cardiomyocytes by any of the treatments. (C) The maximal velocity of shortening ($+dL/dt$) was reduced under hypoxia condition. (D) The maximum velocity of relaxation ($-dL/dt$) was impaired with hypoxia treatment. (E) Time to 90% relengthening (TR90) was increased under hypoxic stress. Values are means \pm SEM, n= 80-110 cells per group, * $p<0.05$ vs. normoxia, respectively; and † $p<0.05$ vs. young hypoxia.



Suppl Figure 2. Rescue of impaired Sesn2 level in aged heart reduces oxidative stress during I/R.
Immunoblots of 4-Hydroxyneonenal (HNE) adduct products and p-Shc⁶⁶ in young, aged and aged+AAV-Sesn2 hearts under sham operations or ischemia/reperfusion (I/R) conditions.

Suppl Table 1. Echocardiographic assessment of cardiac function of WT (young, middle age and aged) and Sestrin2 knockout (Sesn2 KO) mice under normal physiological and ischemia/reperfusion conditions.

Parameter (unit)	Young		Middle age		Aged		Sesn2 KO	
	Basal	I 45'/R 24 h	Basal	I 45'/R 24 h	Basal	I 45'/R 24h	Basal	I 45'/R 24 h
Heart rate (beats.min ⁻¹)	404.0 ± 4.147	417.3 ± 18.53	420.0 ± 11.66	453.0 ± 46.84	425.0 ± 17.49	416.8 ± 28.12	370.0 ± 21.64	410.6 ± 21.03
CO (mL/min)	16.69 ± 1.657	15.41 ± 2.294	16.39 ± 2.685	15.05 ± 3.544	17.27 ± 2.435	13.95 ± 4.299	16.37 ± 3.541	12.44 ± 2.252
IVSd (mm)	0.842± 0.082	0.865 ± 0.063	0.908 ± 0.094	0.943 ± 0.044	0.932 ± 0.073	0.965 ± 0.029	0.86 ± 0.127	0.848 ± 0.062
IVSs (mm)	1.293 ± 0.061	1.288 ± 0.103	1.468 ± 0.023	1.347 ± 0.127	1.455 ± 0.112	1.18 ± 0.064	1.3 ± 0.1455	1.117 ± 0.1091
LVPWd (mm)	0.793 ± 0.064	0.812 ± 0.047	0.793 ± 0.073	0.91 ± 0.161	0.877 ± 0.065	0.712 ± 0.049	0.75 ± 0.047	0.79 ± 0.527
LVPWs (mm)	1.17 ± 0.125	1.01 ± 0.049	1.268 ± 0.068	1.323 ± 0.125	1.328 ± 0.069	0.925 ± 0.087	1.325 ± 0.094	0.987 ± 0.074
LVIDd (mm)	3.935± 0.176	4.22 ± 0.128	3.898 ± 0.228	4.973 ± 0.142 [#]	4.392 ± 0.181	4.485 ± 0.148	3.805 ± 0.170	4.44 ± 0.086
LVIDs (mm)	2.632 ± 0.140	3.20 ± 0.131	2.543 ± 0.248	3.78 ± 0.261 [#]	3.013 ± 0.158	3.715 ± 0.261	2.49 ± 0.166	3.615 ± 0.141
EF (%)	62.18 ± 1.664	52.36 ± 0.957 [#]	59.98 ± 2.279	41.82 ± 1.82 [#]	58.31 ± 2.98	31.17 ± 1.64 ^{#,†}	62.29 ± 2.35	31.45 ± 2.16 ^{#,†}
FS (%)	33.88 ± 0.953	25.33 ± 0.86 [#]	31.68 ± 1.53	19.62 ± 0.52 [#]	30.8 ± 2.04	14.92 ± 0.63 ^{#,†}	33.67 ± 1.64	16.44 ± 1.30 ^{#,†}
Body weight (g)	26.75 ± 2.17		36.22 ± 1.61*		37.65 ± 3.23*		26.98 ± 2.40	

Values are means±SEM; n=4-6; [#]p<0.05 vs. basal; *p<0.05 vs. young basal; †p<0.05 vs. young I/R.

Suppl Table 2. Working heart function of WT (young and aged) and Sestrin2 knockout (Sesn2 KO) mice under basal and ischemia/reperfusion conditions.

Parameter (unit)	Young		Aged		Sesn2 KO	
	Basal	I/R	Basal	I/R	Basal	I/R
Systolic pressure (mmHg)	84.38 ± 3.94	41.06 ± 2.81*	81.96 ± 5.06	33.88 ± 3.34*	80.05 ± 4.86	32.95 ± 3.29*
Diastolic pressure (mmHg)	22.39 ± 2.57	19.52 ± 2.71	24.28 ± 2.90	19.27 ± 1.35	23.37 ± 2.53	19.05 ± 2.78
CO (ml/min)	8.55 ± 0.35	3.70 ± 0.24*	7.73 ± 0.35	2.72 ± 0.47*	7.83 ± 0.42	2.70 ± 0.43*
Coronary flow (ml/min)	2.11 ± 0.21	1.18 ± 0.19*	2.02 ± 0.10	0.89 ± 0.06*	2.11 ± 0.11	0.78 ± 0.08*

Values are means±SEM; n=5-6; *p<0.05 vs. basal.

Suppl Table 3. Echocardiographic assessment of cardiac function of aged mice with AAV viral delivery under normal physiological and ischemia/reperfusion conditions.

Parameter (unit)	Young		Aged		Aged-AAV-Scram		Aged AAV-Sesn2	
	Basal	I 45'/R 24 h	Basal	I 45'/R 24 h	Basal	I 45'/R 24h	Basal	I 45'/R 24 h
Heart rate (beats.min ⁻¹)	404.0 ± 4.147	417.3 ± 18.53	425.0 ± 17.49	416.8 ± 28.12	428.3 ± 6.408	418.8 ± 13.82	417.0 ± 18.89	420.0 ± 15.67
CO (mL/min)	16.69 ± 1.657	15.41 ± 2.294	17.27 ± 2.435	13.95 ± 4.299	16.93 ± 1.906	13.41 ± 1.807	17.50 ± 3.795	15.27 ± 2.312
IVSd (mm)	0.842 ± 0.082	0.865 ± 0.0629	0.932 ± 0.073	0.965 ± 0.029	0.978 ± 0.54	1.018 ± 0.043	0.89 ± 0.072	0.816 ± 0.072
IVSs (mm)	1.293 ± 0.061	1.288 ± 0.103	1.455 ± 0.112	1.18 ± 0.064	1.545 ± 0.064	1.235 ± 0.064	1.242 ± 0.120	0.994 ± 0.0796
LVPWd (mm)	0.793 ± 0.064	0.812 ± 0.047	0.877 ± 0.065	0.712 ± 0.049	0.85 ± 0.065	0.825 ± 0.042	0.96 ± 0.071	0.824 ± 0.025
LVPWs (mm)	1.17 ± 0.125	1.01 ± 0.049	1.328 ± 0.069	0.925 ± 0.087	1.27 ± 0.083	0.973 ± 0.0320	1.244 ± 0.077	1.112 ± 0.053
LVIDd (mm)	3.935 ± 0.176	4.22 ± 0.128	4.392 ± 0.181	4.485 ± 0.148	4.32 ± 0.365	4.343 ± 0.115	4.188 ± 0.163	4.32 ± 0.145
LVIDs (mm)	2.632 ± 0.140	3.20 ± 0.131	3.013 ± 0.158	3.715 ± 0.261	2.925 ± 0.273	3.645 ± 0.142	3.02 ± 0.149	3.564 ± 0.138
EF (%)	62.18 ± 1.664	52.36 ± 0.957#	58.31 ± 2.98	31.17 ± 1.64#†*	63.98 ± 1.52	30.00 ± 2.60#†*	60.98 ± 1.573	41.88 ± 2.06#†
FS (%)	33.88 ± 0.953	25.33 ± 0.86#	30.8 ± 2.04	14.92 ± 0.63#†*	34.68 ± 0.90	14.39 ± 1.07#†*	32.40 ± 0.282	22.83 ± 0.57#

Values are means±SEM; n=4-6; #p<0.05 vs. basal; *p<0.05 vs. Aged AAV IR; †p<0.05 vs. young I/R.